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SYNTHESIS OF ^{14}C-LABELLED OCTAHYDRO-1,3,5,7-TETRANITRO-1,3,5,7-TETRAZOCINE (HMX) AND ^{15}N-ISOTOPIC HEXAHYDRO-1,3,5-TRINITRO-1,3,5-TRIAZINE (RDX) FOR USE IN MICROCOSM EXPERIMENTS

by

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SANS CLASSIFICATION

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ABSTRACT

Within the framework of an R & D project on the bioremediation of soils contaminated with energetic compounds, the biodegradation of energetic nitramine products such as octogen (HMX) and hexogen (RDX) is under study. Microcosm assays can be performed with radioactive carbon-14 labelled substrates or with isotopic nitrogen-15 labelled substrates in order to ascertain and monitor their biodegradation pathways and to optimize parameters for a bioremediation process. To synthesize ^{14}C-HMX, acetylation of labelled hexamethylenetetramine (^{14}C-HMTA) was done yielding 3,7-diacetyl-1,3,5,7-tetraazabicyclo-[3.3.1]-nonane (^{14}C-DAPT) which was nitrated to obtain 1,5-diacetyloctahydro-3,7-dinitro-1,3,5,7-tetrazocine (^{14}C-DADN) in one step. Nitrolysis of ^{14}C-DADN was achieved using a mixture of 100% nitric acid and phosphorus pentoxide to yield ^{14}C-HMX. The synthesis of this carbon-14 labelled HMX was optimized first using cold starting materials and then conducted with labelled compounds. To synthesize ^{15}N-RDX, nitration of nitrogen-15 hexamethylenetetramine (^{15}N-HMTA) was done according to the Hale process. ^{15}N-HMTA was prepared by reacting cold formaldehyde with isotopic nitrogen-15 ammonium hydroxide.

RÉSUMÉ

Dans le cadre d'un projet de recherche et développement sur la biorestauration de sols contaminés par les matériaux énergétiques, la biodégradation des nitramines énergétiques tel que l'octogène (HMX) et l'hexogène (RDX) est présentement à l'étude. Des essais en microcosme peuvent être effectués avec des substrats radioactifs marqués au carbone 14 ou encore marqués isotopiquement à l'azote 15, dans le but d'établir et de suivre les mécanismes de biodégradation et d'optimiser les paramètres du procédé de biorestauration. Pour synthétiser le HMX-^{14}C, l'acétylation de l'hexaméthylènetétramine marquée (HMTA-^{14}C) a été faite pour obtenir le 3,7-diacétyl-1,3,5,7-tétraazabicyclo-[3.3.1]-nonane (DAPT-^{14}C) qui a ensuite été nitré pour produire la 1,5-diacétyloctahydro-3,7-dinitro-1,3,5,7-tétrazocine (DADN-^{14}C) en une étape. La nitrolyse du DADN-^{14}C a été effectuée en utilisant un mélange d'acide nitrique 100 % et du pentoxyde de phosphore pour produire le HMX-^{14}C. La synthèse de ce HMX marqué au carbone 14 a été optimisée en utilisant d'abord des réactifs froids, puis les produits de départ radioactifs. Pour synthétiser le RDX-^{15}N, on a effectué la nitration de l'hexaméthylènetétramine marquée à l'azote 15 (HMTA-^{15}N) selon le procédé de Hale. L'hexaméthylènetétramine HMTA-^{15}N a été obtenue en faisant réagir de la formaldéhyde froide avec de l'hydroxyde d'ammonium marqué à l'azote 15.

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EXECUTIVE SUMMARY

The international context of demilitarization, increasing environmental importance of human activities, the closure of military bases, the increasing demand for new technologies for the clean-up of sites and the more severe aspects of the law to protect the environment, have led to the establishment of new areas for research and development. Many activities of the Canadian Forces such as firing, demolition and destruction of obsolete ammunition by open burning and open detonation may lead to the dispersion of energetic compounds in the environment. In Canada, limited effort has been spent to examine this particular environmental threat. Energetic compounds are just now being recognized as environmental contaminants as compared with other contaminants such as petroleum or solvents. Energetic compounds are unique due to their highly specific physical, chemical and toxicological properties.

It is within this context that the Defence Research Establishment Valcartier (DREV) initiated an R&D program in 1991 to study the environmental impact of energetic materials that are found in the DND ammunition stockpile. Another aspect of DREV's R&D program is the development of practical and economical remediation technologies for the cleaning of explosives-contaminated sites. Both programs on soil characterization and soil remediation will position the Department of National Defence in a state of readiness for any future potential contamination problems. DREV decided to develop biotechnologies as a remediation technique because these technologies are cheap, efficient, innovative and accepted by the public. To achieve that goal, DREV initiated a program with the Biotechnology Research Institute of the National Research Council of Canada to match their strong expertise in biotechnology with DREV's expertise in energetic materials. The research program consisted in identifying indigenous microorganisms capable of biodegrading energetic materials into less or ideally non-toxic materials.

To understand the biodegradation pathway and the mechanisms of biodegradation, microcosm experiments are performed with radioactive compounds. When biodegradation occurs, carbon dioxide is formed and trapped by a sodium hydroxide solution contained in the microcosm. It is difficult to quantify carbon dioxide chemically, but radioactive carbon dioxide is very easy to detect and quantify. For this reason, it is imperative to use radioactive target molecules in microcosm experiments to study their biodegradation. Since radioactive explosives are not available commercially, DREV synthesized radioactive explosives to study their biodegradation pathway. To synthesize carbon-14 HMX, all possible synthetic scenarios were evaluated with cold materials to choose the best route with high yields and low radioactive waste. The best synthesis is described in this report. Furthermore, to better understand the biodegradation of the target explosives, nitrogen-15 compounds had to be synthesized. All microorganisms need and use carbon and nitrogen sources as energy sources and, understanding how nitrogen is retrieved from the explosives and where it is going can reveal important pieces of the biodegradation puzzle. For this reason, it was decided to investigate the synthesis of nitrogen-15 RDX, which is also described in this report.

NOMENCLATURE

$\alpha,\beta,\delta,\gamma$	Alpha, Beta, Delta, Gamma
BRI	Biotechnology Research Institute
CM^{-1}	Wavelength per centimeter
DADN	1,5-Diacetyloctahydro-3,7-Dinitro-1,3,5,7-Tetrazocine
DANNO	1,5-Diacetyloctahydro-3-Nitro-7-Nitroso-1,3,5,7-Tetrazocine
DAPT	3,7-Diacetyl-1,3,5,7-Tetraazabicyclo-[3.3.1]-Nonane
DND	Department of National Defence
DREV	Defence Research Establishment Valcartier
GAP	Glycidyl Azide Polymer
$^{2}H, ^{15}N, ^{13}C, ^{18}O$	Deuterium, nitrogen-15, carbon-13, oxygen-18
HMTA	Hexamethylenetetramine
HMX	Octogen or 1,3,5,7-Tetranitro-1,3,5,7-Tetrazocine
IR	Infrared Spectroscopy
KBr	Potassium Bromide
MHz	Megahertz
MCi	Millicurie
Mmole	Millimole
M.P.	Melting Point
NC	Nitrocellulose
NMR	Nuclear Magnetic Spectroscopy
ppm	Parts per million
RDX	Hexogen or 1,3,5-Trinitro-1,3,5-Triazine
R&D	Research and Development
TAT	1,3,5,7 Tetraacetyloctahydro-1,3,5,7-Tetrazocine
TNT	Trinitrotoluene
γ_{max}	Maximum vibration frequency

1.0 INTRODUCTION

During the last decade, many needs have already emerged with respect to identification, quantification, delimitation and elimination of energetic contaminants dispersed by munitions or, present in explosives dumps, trials or destruction fields, firing areas and production sites (Refs. 1-2). Possible solutions for site decontamination include recycling, chemical treatment, incineration, or stabilization/encapsulation. Biotechnologies are currently being considered as viable treatment options for bioremediation of sites contaminated by explosives. These technologies utilize microorganisms to mineralize or to biodegrade organic contaminants into less hazardous compounds (Refs. 3-4). Bioremediation has the advantage of being less expensive than incineration and is acceptable to the public. Within this context, a feasibility study was initiated on the biodegradation of selected energetic materials by microorganisms enriched from contaminated soils and a bioremediation biopile process was set up to mineralize RDX and HMX in contaminated soils (Refs. 5-12). Before achieving bioremediation, the characterization of the contaminated sites was studied and appropriate sampling procedures were developed (Refs. 13-15).

To identify microorganisms capable of biodegrading or mineralizing explosives, laboratory-scale microcosm studies can be performed with ^{14}C-labelled explosives that represent the key products allowing the mineralization process to be monitored by the evolution of ^{14}C-carbon dioxide. Since radioactive explosives are not generally available commercially, it was necessary to synthesize ^{14}C-labelled-RDX, TNT, NC, GAP (Ref. 16) and ^{14}C-HMX for such a study. One should keep in mind that when synthesizing radioactive explosives, safety is of great concern to avoid personnel injury and radioactive contamination problems. Moreover, high yield routes must be used to avoid the disposal of radioactive explosive wastes and to optimize the use of expensive carbon-14 labelled starting materials. In order to achieve this, a safe and high yield synthetic route to ^{14}C-HMX was required. Moreover, during preliminary experiments to biodegrade RDX, it was found that RDX was used as a nitrogen source. To understand how nitrogen was retrieved from RDX and where it was going during the biodegradation, it was decided to synthesize ^{15}N-RDX to study the pathway of nitrogen consumption into biodegradation. This work was carried out at DREV between April

1995 and June 1998 under work unit 2ng14 "Development of Bioremediation technologies". This report describes the synthesis of ^{14}C-labelled HMX and of ^{15}N-RDX.

2.0 THEORY

Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX, for High Melting Explosive) also called, octogen, homocyclonite and cyclotetramethylenetetramine, is the most powerful non-atomic explosive in military use and is much more expensive than RDX (Ref. 17). It is used as a high explosive in octol mixtures which are comprised of 70% HMX and 30% TNT. When malfunction occurs with ammunition containing octol, the casing breaks into pieces upon impact with the target and spreads the octol mixture on the ground leading to soil and groundwater contamination by HMX and TNT. This situation was frequently encountered on anti-tank ranges and is described in Refs. 13-14. To synthesize ^{14}C-HMX, many routes such as: the Bachmann process, the TAT, the DADN and the DANNO procedures were evaluated to determine the best way to obtain HMX in high yield and purity. Organic contaminants are often used as nitrogen sources by microorganisms. To identify the pathway of biodegradation, one could use nitrogen-15 labelled target molecules. These nitrogen-15 molecules are biotransformed or mineralized and the introduction of nitrogen into the biomass can be followed to better understand the biodegradation pathway. Usually, NMR spectroscopy or mass spectrometry is used to follow the ingestion of nitrogen-15. To better understand the mineralization of RDX which is used as a nitrogen source in the biodegradation process, ^{15}N-RDX was synthesized and used in microcosm experiments.

2.1 Synthesis of ^{15}N-RDX

The easiest and oldest process consists in introducing hexamethylenetetramine (HMTA) into concentrated nitric acid at 25-30°C. Hale noted that the nitration must be done in a 4 to 8 fold excess of nitric acid and this process was chosen to synthesize ^{14}C-RDX in a previous work (Refs. 16 and 18). At the end of the reaction, RDX is isolated by precipitation in cold water. A complete description the available synthetic routes for the synthesis of RDX, including the Bachmann process, can be retrieved from the literature (Ref.16). The Hale process was also chosen to synthesize our

nitrogen-15 RDX since the method is simple and gives good yields. Nitrogen-15 RDX was synthesized because it was found that microorganisms used RDX as a nitrogen source in the biodegradation process. Mass spectroscopy and NMR spectroscopy can be used to trace the nitrogen-15 and understand if, for example, the nitrogen is incorporated into the biomass or if it stays in the soils. The use of nitrogen-15 compounds combined with experiments performed with carbon-14 compounds helps in solving the biodegradation pathway since it gives more complementary information.

2.2 The Bachmann Process

The synthetic route for the industrial production of HMX is the process developed by Bachmann and Sheehan which was later modified by Castorina *et al.* (Refs. 19-20). In this synthesis, hexamethylenetetramine is reacted with nitric acid in the presence of ammonium nitrate and acetic anhydride. Even if the starting materials are cheap, producing HMX with this process is much more expensive than producing RDX since the production rate is slow. Moreover, the Bachmann process suffers from poor yield and gives a mixture of HMX/RDX containing 73% HMX which was increased to 85% using paraformaldehyde (Refs. 20-21). HMX exists in 4 polymorphic forms (α, β, γ and δ) between room temperature and its melting point. The β form is the one used for military applications. One could isolate pure β-HMX from the HMX/RDX mixture obtained by the Bachmann process by placing the crude product in a solution of sodium tetraborate decahydrate in water. Boiling and adding a 5N sodium hydroxide soluition until the pH increases above 9.7 destroys the RDX and allows recovery of HMX, which could be recrystallized from nitromethane (Ref. 17). Separation of the HMX/RDX mixture could also be performed by dissolving the RDX in ethylene dichloride leaving a HMX residue that is recrystallized twice from acetone and, RDX is recovered from the ethylene dichloride extract to be recrystallized twice from 50% aqueous acetone. Bulusu *et al.* used the Bachmann process and this technique to prepare RDX and HMX labelled with isotopes such as: ^{2}H, ^{15}N, ^{13}C and ^{18}O (Refs. 22-24). Since they were interested in obtaining isotopic RDX and HMX, this process was suitable for their needs. In this study, our goal was to synthesize ^{14}C-HMX with a high yield avoiding purification procedures or separation to minimize possible contamination and the generation of radioactive wastes.

The synthetic routes developed by Gilbert *et al.* seemed more attractive to us since they claimed high yields for the steps involved in HMX synthesis (Ref. 25). In the study by Gilbert and co-workers, three routes were investigated and described as the TAT, the DADN and the DANNO procedures. The TAT and DADN routes are illustrated in Fig. 1.

2.3 The TAT Procedure

The first route studied by Gilbert *et al.* is called the TAT procedure and consists in acetylating hexamethylenetetramine to prepare 3,7-diacetyl-1,3,5,7-tetraazabicyclo-[3.3.1]-nonane (DAPT) in a quantitative yield (Ref. 26). Acetylation of DAPT yields 1,3,5,7-tetraacetyloctahydro-1,3,5,7-tetrazocine (TAT) (89%). Nitrolysis of TAT using nitric acid-phosphorous pentoxide yields HMX (77%) when a mixture containing 40 parts of phosphorous pentoxide/ 100 parts of 96% nitric acid is used (Ref. 25). It was also found by Lukasavage *et al.* that TAT can be transformed easily into 100% pure HMX with a 99% yield using a mixture similar to the one used by Gilbert. Lukasavage *et al.* used a mixture of nitric acid 98% (500g) and phosphorous pentoxide (70g) to nitrolyse TAT at room temperature (Ref. 27). Since HMTA and DAPT could be synthesized quantitatively (Ref. 16, 26), that TAT could be obtained from DAPT (89% yield) and be nitrolysed to HMX (100%), this route was investigated with cold products.

HMTA was obtained with a quantitative yield and used without further purification for the preparation of the other HMX precursors. This product was identical to the one isolated in Ref. 16. The DAPT synthesis yielded, after recrystallization, a pure product (92%, m.p. 190-192°C, lit. 192°C, Ref. 26). The spectroscopic analysis of this product was identical to the one described in Ref. 26. Wamhoff *et al.* used a mixture of thionyl chloride, ammonium acetate and traces of water in acetic anhydride to acetylate DAPT to yield TAT (53%), after recrystallization in ethanol (Ref. 28). According to the procedure of Gilbert, we used a mixture of acetyl chloride, acetic anhydride, glacial acetic acid and sodium acetate to acetylate DAPT. Crude TAT was isolated and recrystallized from a mixture of benzene/ethanol to yield a pure sample (92%) with a m.p. of 157-158°C (lit. 153-158 °C, Ref. 25). The spectroscopic analysis of this product was identical to the one described by Coon *et al.* (Ref. 29).

The nitrolysis of this TAT sample was performed using the procedure described by Lukasavage *et al.* (Ref. 27). All attempts to reproduce the yield claimed in this process, failed and only very poor yields were obtained. Even when the reaction was carried out under a glove box to avoid moisture, yields exceeding 20% were never achieved. Compared to the nitrolysis conditions proposed by Gilbert, the Lukasavage procedure involves almost the same nitrolysis system based on a mixture of 98% nitric acid/phosphorus pentoxide but at room temperature and for a longer time. Gilbert *et al.* used a mixture of 96% nitric acid/phosphorus pentoxide at 75°C for 15 minutes. As stated by Gilbert, it is highly probable that the strength of nitric acid has a direct effect on the yield of the reaction. The temperature is also a very important parameter. Gilbert mentioned that lowering the temperature below 75°C in the nitrolysis of DADN gave poor yields. Moreover, it is seen in reference 25 that, when the proportion of phosphorus pentoxide in the mixture is between 10 and 20%, even with a larger quantity of nitric acid, the yields obtained for the conversion of TAT to HMX are between 7-31%. Since Lukasavage reported a 100% yield with 14% phosphorus pentoxide in his nitrolysis mixture, it is seen that these results are contradictory.

Since all of our nitrations were performed using fuming nitric acid or red fuming nitric acid and since we were not able to reproduce the Lukasavage process, it was decided to perform all the nitrations again using freshly distilled nitric acid (100%). Using 100% nitric acid resulted in a high yield for the conversion of TAT to HMX. Reproducing the Lukasavage conditions (14% phosphorous pentoxide in 100% nitric acid) resulted in the production of HMX containing small quantities of DADN (67-80% yield). Increasing the proportion of phosporous pentoxide to 35% using the same conditions resulted in the production of pure HMX (86%). Therefore, it is seen that the strength of the nitric acid, the concentration of phosphorus pentoxide and the temperature of the reaction are critical parameters in these nitrations. In this route, assuming that the yield for HMTA is quantitative, the yields for DAPT and TAT are both 92% and that the conversion of TAT to HMX gives a yield of 86%, the overall conversion from formaldehyde to HMX would have been 73%, a lower overall yield than the Bachmann process (82% yield, HMX/RDX 73:27, Ref. 30). This route involves more steps than the Bachmann process and the overall yield is poorer so this route was abandoned.

2.4 The DADN Procedure

The second route proposed by Gilbert is called the DADN procedure and involves nitration of DAPT to yield 1,5-diacetyloctahydro-3,7-dinitro-1,3,5,7-tetrazocine (DADN). It was found that DADN could be synthesized directly from HMTA in one step without isolating DAPT (Ref. 25). The nitrolysis of DADN to HMX could then be performed using seven different nitrolysis systems with yields varying from 44 to 99%. Among them, two systems give 99% yields with 100% purity. Our efforts concentrated on these two systems which involved mixtures of nitric acid/polyphosphoric acid or mixtures of nitric acid/phosphorus pentoxide. This route, illustrated in Fig. 1, was scaled up by Gilbert and proved to be a viable alternative to the Bachmann process. Although it was found the nitrolysis of DAPT is exothermic, it could be suppressed by using urea (Ref. 31). A recent study has proposed several alternatives to the use of urea as an exotherm suppressant (Ref.32).

The acetylation and nitration of HMTA was performed according to the procedure of Gilbert *et al.* to yield DADN (95%, m.p: 265°C). The spectroscopic analysis of this product was in accordance with the reported analysis (Ref. 25). Nitrolysis of DADN was performed using, a 100% nitric acid/polyphosphoric acid system at a ratio of 7.5 g/16.5 g per gram of DADN, and HMX was obtained at yields varying from 69 to 84%. Shackelford *et al.* also used the nitric acid/polyphosphoric acid system to nitrolyse DADN and mentioned that a lot of precautions were required for the synthesis. They obtained yields similar to ours for this nitrolysis (Ref. 33). Nitrolysis of DADN was also performed using a 100% nitric acid/phosphorus pentoxide system at a ratio of 25 g/8.4 g per gram of DADN, and HMX was obtained at high yields (90-94%). Therefore, considering that DADN was synthesized directly from HMTA at 95% yield and, that DADN was easily nitrolysed to HMX with a yield of 94%, the overall yield for HMX synthesis is 85%, which is superior to the Bachmann process. This synthetic route was chosen for the synthesis of ^{14}C-HMX using the 100% nitric acid/ phosphorus pentoxide system.

2.5 The DANNO Procedure

The third route proposed by Gilbert *et al.* showed that DAPT could be converted in 1,5-diacetyloctahydro-3-nitro-7-nitroso-1,3,5,7-tetrazocine (DANNO) by using a mixture of nitric acid/ nitrogen dioxide or red fuming nitric acid for about a 84-88% yield. This is a lower yield than for the DADN synthesis from HMTA, but it has the advantage of not using sulfuric acid in the production process. The nitrolysis of DANNO to HMX could be performed with a three-stage process using nitric acid for 15 minutes followed by the addition of polyphosphoric acid for an additional hour, then more nitric acid was added in the last stage to yield HMX (86-92%). It was concluded that HMX was formed in lower yield and degree of purity from DANNO was less than that from DADN, and that the process is less thorough. For these reasons, this route was not considered for ^{14}C-HMX synthesis.

3.0 EXPERIMENTAL

3.1 Chemical Suppliers

All solvents were purchased from Fisher Scientific Co., except anhydrous ethanol, which was obtained from "Les alcools de commerce limitée". Chemicals and starting materials such as aqueous formaldehyde and phosphorus pentoxide were purchased from Aldrich Chemical Co. The acids and bases were purchased from Baker except for the fuming nitric acid and red fuming nitric acid which were purchased from Aldrich Chem. Co. Fuming sulfuric acid (20% oleum) was purchased from ACP Chem. Co. Finally, ^{14}C-formaldehyde (58 mCi/mmol, 1mCi/mL) and ^{15}N-ammonium hydroxide was obtained respectively from Sigma Chemical. Co. and Isotech Inc.

3.2 IR and NMR Spectroscopy

Infrared absorption spectra were registered with a Perkin-Elmer IR spectrophotometer (model 580) or with a Digilab FTS-40 spectrophotometer combined with a DTGS detector. The infrared spectra for the solids were recorded using solid suspensions in KBr. ^{1}H and ^{13}C Nuclear

magnetic resonance spectra were obtained from a Varian Gemini spectrometer (200 MHz). Spectra were recorded using deuterated chloroform or deuterated dimethyl sulfoxide as the solvent and tetramethylsilane as the internal reference at 0.0 ppm on the δ scale.

3.3 Cold Syntheses of HMX

To identify the best route for the synthesis of ^{14}C-HMX, preparations of HMTA, DAPT, DADN, TAT and HMX were performed using cold starting materials according to known procedures. The yields were in agreement with those reported in the literature (Refs. 25-29, 33). HMTA was obtained quantitatively, DAPT was obtained from HMTA with a yield of 92% (m.p.: 190-192°C, lit m.p.:192°C, 193-195°C, Refs. 26, 34). The spectroscopic analysis for this product is as follows: (IR: υ_{max} (KBr) cm^{-1}: 3420, 3000, 2920, 2880, 1625, 1480, 1460, 1430, 1385, 1380, 1350, 1320, 1310, 1210, 1190, 1080, 1020, 990, 970, 950, 940, 830, 810, 780, 680, 640, 600, 590, 580, 510, 480, 390. ^{1}H NMR: δ ($CDCl_3$) ppm : 5.64 (d, 2H, J=15 Hz, NCH_AH_BNAc), 4.76 (AB system, 4H, j=15Hz, NCH_AH_BNAc), 4.14 (s, 2H, NCH_2N), 4.13 (d, 2H, j=15 Hz, the other two NCH_AH_BNAc), 1.98 (s, 6H, CH_3). ^{13}C NMR: δ ($CDCl_3$) ppm: 169.9 (CO, acetyl), 71.1 (NCH_2N), 67.5 (two NCH_2NAc) 62.9 (the two other NCH_2NAc), 21.9 (CH_3).

DADN was synthesized directly from HMTA (95% yield) while TAT was prepared from DAPT at a 92% yield. The melting point of TAT was 157-158°C (lit m.p.: 153-158°C, Ref. 25) and the spectroscopic analysis was as follows: IR: υ_{max} (KBr) cm^{-1}: 3420, 3020, 2995, 2940, 1650, 1490, 1420, 1380, 1340, 1300, 1260, 1230, 1210, 1180, 1110, 1060, 1040, 980, 940, 920, 820, 780, 620, 580, 540, 490. ^{1}H NMR: δ ($CDCl_3$) ppm: 5.08 (s, 8H, CH_2), 2.32 (s, 12H, CH_3). ^{13}C NMR: δ ($CDCl_3$) ppm: 172.2 (CO, acetyl), 59.5 (CH_2), 22.9 (CH_3).

HMX was synthesized from TAT and from DADN using either nitric acid/polyphosphoric acids or nitric acid/phosphorus pentoxide system for the nitrolysis of HMX obtaining yields varying from 67 to 94%. The parameters for the nitrolysis were in accordance with the Lukasavage process or the Gilbert procedure (Refs. 25 and 27). The best results obtained with the nitric acid/phosphorus

pentoxide conditions were used for ^{14}C-HMX synthesis and are described. Nitric acid 100% was obtained by distilling concentrated nitric acid in the presence of concentrated sulfuric acid.

3.4 Synthesis of ^{14}C-HMX

3.4.1 ^{14}C-Hexamethylenetetramine (^{14}C-HMTA)

^{14}C-Formaldehyde (2 mCi, 58 mCi/mmole, 2 x 1mCi/mL) was quantitatively transferred into a three-neck flask (25 mL) containing unlabelled aqueous formaldehyde (37%) (5.92g, 0.0729 mole) and the flask was immersed into a water bath at 18°C. Concentrated ammonium hydroxide (3.8mL) was added dropwise for 30 minutes in order to maintain the temperature between 28 and 35°C. This solution was heated at 33-35°C for 24 hours and then transferred to a flask (250 mL) into which water was added to precipitate the product. The glassware was thoroughly washed to avoid contamination and the rinse water was added to the flask. Water was evaporated to yield ^{14}C-HMTA (1.70 g, quantitative, mp: 268-272°C, decomposes). IR: υ_{max} (KBr) cm^{-1}: 2960, 2940, 2880, 1460, 1440, 1370, 1240, 1000, 810, 670. ^{1}H NMR : δ ($CDCl_3$) ppm : 4.73 (all protons, s). ^{13}C NMR: δ ($CDCl_3$) ppm: 74.8 (all carbons).

3.4.2 ^{14}C-1,5-Diacetyloctahydro-3,7-Dinitro-1,3,5,7-Tetrazocine (^{14}C-DADN)

In a three-neck flask (25 mL) equipped with a reflux condenser and a magnetic stirrer, ^{14}C-HMTA (1.5936 g, 0.0113 mole) and ammonium acetate (0.7044 g, 0.0091 mole) were added to water (0.7968 g) at 5-10°C. Acetic anhydride (3.48 g, 0.0340 mole) was added dropwise to this stirred solution over 60 minutes at 5-10°C. This DAPT solution was stirred at 5-10°C for an additional 30 minutes and transferred to an addition funnel. This solution was added dropwise over 80 minutes to a vigorously mechanically stirred mixture of 94% fuming nitric acid (7.17 g, 0.1052 mole) and 96% fuming sulfuric acid 20% oleum, (25.15 g, 0.2412 mole) at 18-20°C. After stirring for 20 minutes at 20°C, the solution was mixed with ice (230 g). The product precipitated with dilution with another 250 mL water. The product was filtered, washed and dried to yield ^{14}C-DADN (2.889 g, 0.0099 mole, 88% yield, m.p: 265°C, lit. 265°C, ref.24). IR: υ_{max} (KBr) cm^{-1}: 3400, 3020,

2940, 1675, 1520,1470, 1440, 1420, 1400, 1370, 1340, 1270, 1180, 1120, 1080, 1020, 990, 970, 940, 860, 810, 790, 760, 730, 620, 585, 510, 400, 390. ^{1}H NMR: δ (DMSO-$_{D6}$) ppm: 5.54 (m, 8H, methylene), 2.27 (S, 6H, CH_3). ^{13}C NMR: δ (DMSO-$_{D6}$) ppm: 165.0 (CO, acetyl), 61.9, 59.8 (CH_2), 21.6 (CH_3).

3.4.3 ^{14}C-Octahydro-1,3,5,7-Tetranitro-1,3,5,7-Tetrazocine (^{14}C-HMX)

In a three-neck flask (250 mL), equipped with a mechanical stirrer and containing a mixture of 100% nitric acid (72.25 g, 1.15 mole) and phosphorus pentoxide (24.3 g, 0.0855 mole) ^{14}C-DADN (2.89 g, 0.0099 mole) was added. This solution was stirred at 50°C for one hour and then at room temperature for 20 hours. The solution was poured onto crushed ice (200 g) and the product was precipitated by adding water (800 mL). The suspension was kept at -4°C overnight and then the product was filtered, washed and air dried to yield pure ^{14}C-HMX (2.68 g, 0.0090 mole, 91%, m.p: 274-276°C, lit: m.p: 276-280°C decomposes, Ref. 16). This product had an activity of 675 μCi. IR: υ_{max} (KBr) cm^{-1}: 3436, 3036, 2960, 1565, 1465, 1435, 1395, 1348, 1279, 1203, 1145, 1087, 965, 946, 830, 759, 658, 626, 600. ^{1}H NMR: δ (DMSO-$_{D6}$) ppm: 6.03 (S, all protons). ^{13}C NMR: δ (DMSO-$_{D6}$) ppm: 67.2 (all carbons).

3.5 Synthesis of ^{15}N-RDX

3.5.1 ^{15}N-hexamethylenetetramine (^{15}N-HMTA)

Unlabelled aqueous formaldehyde (37%) (31.36g, 0.3864 mole) was introduced into a three-neck flask (100 mL) immersed into a water bath at 18°C. Concentrated ^{15}N-ammonium hydroxide (20 mL) was added dropwise for 30 minutes in order to maintain a temperature between 28 and 35°C. This solution was heated at 33-35°C for 24 hours and then transferred to a flask (1 L) where water was added to precipitate the product. The glassware was thoroughly washed to avoid contamination and the rinse water was then added to the flask. Water was evaporated to yield ^{15}N-HMTA (9.01 g, quantitative, mp: 265-272°C (decomp)). IR: υ_{max} (KBr) cm^{-1}: 2960, 2940, 2880,

1460, 1440, 1370, 1240, 1000, 810, 670. ^{1}H NMR: δ ($CDCl_3$) ppm: 4.73 (all protons,s). ^{13}C NMR: δ ($CDCl_3$) ppm: 74.83 (all carbons).

3.5.2 ^{15}N-Hexahydro-1,3,5-trinitro-1,3,5-triazine (^{15}N-RDX)

In a three-neck flask (500 mL), equipped with a reflux condenser and a magnetic stirrer, fuming nitric acid (157.6 g) was cooled at 5°C and ^{15}N-HMTA (9.01 g, 0.064 mole) was added slowly over a period of 1 hour in order to keep the mixture temperature between 5-10°C. The temperature of the solution was then increased to 15-20°C and held there for 3 hours. The solution was poured into a beaker containing water (160 mL) to precipitate the ^{15}N-RDX. The solid was filtered, washed thoroughly with water, with a sodium carbonate solution (5%) and again with water until neutral pH was achieved. The RDX was dried at room temperature for 2 days. Dried ^{15}N-RDX was collected (8.0 g, 0.036 mole, 56% yield) (mp: 201-208°C, lit. 202°C, ref.15, lit. 204°C, ref.6) and flegmatized with ethanol (8%). IR: υ_{max} (Acetone) cm^{-1}: 3070, 1590, 1310, 1270, 1010, 930-920, 790. ^{1}H NMR: δ ($CDCl_3$) ppm: 6.01 (all protons). ^{13}C NMR: δ ($CDCl_3$) ppm: 60.31 (all carbons).

4.0 RESULTS AND DISCUSSION

Macro amounts of ^{14}C-HMX were prepared by mixing the available labelled starting material (^{14}C-formaldehyde) with its unlabelled homologue in sufficient quantity to obtain 2 g of the desired end-product. Since a loss of carbon occurs during the opening of HMTA, radioactivity is lost during this step. Moreover, since the labelled starting materials (^{14}C-Formaldehyde) is mixed with cold formaldehyde to synthesize HMTA, radioactive carbons could be found statistically in all HMX rings that are not fully ^{14}C-labelled.

4.1 Synthesis of ^{14}C-HMX

As discussed, the DADN route was chosen for ^{14}C-HMX synthesis. Since this route first consists in acetylating HMTA, it was necessary to synthesize ^{14}C-HMTA. The synthesis of ^{14}C-

HMTA was performed using commercially available ^{14}C-formaldehyde with ammonium hydroxide and has been reported elsewhere (Ref. 16). As mentioned, ^{14}C-formaldehyde was mixed with unlabelled formaldehyde, the labelled carbons could then be found statistically in HMTA rings, where their possible locations are indicated by dots in Fig. 1. During the acetylation and nitration of HMTA to yield DADN, two methylenes were cleaved. This loss of carbons contributed to a decrease in the total radioactivity of the HMX end-product.

In the current ^{14}C-HMX synthesis, ^{14}C-formaldehyde (2 mCi) was mixed with unlabelled aqueous formaldehyde (37%) and reacted with concentrated ammonium hydroxide to yield ^{14}C-HMTA, quantitatively. Acetylation and nitration of this product yielded ^{14}C-DADN (88%) (mp: 265°C, lit. 265°C, ref.25). Infrared and NMR spectroscopy of the product were identical to the one of a known sample. The yield for this step of the synthesis was similar to that reported in the literature (Ref.25). Nitrolysis of ^{14}C-DADN was performed using freshly distilled 100% nitric acid/ phosphorus pentoxide at 50°C for 60 minutes followed by stirring at 28°C for 20 hours resulting in the production of pure ^{14}C-HMX (91%, m.p. 274°C, lit. 276-278°C, ref. 17). Infrared and NMR spectroscopy of the ^{14}C-HMX product demonstrated that it was identical to that of a commercial sample (Ref. 33). No signals corresponding to DADN, at 5.5 and 2.3 ppm, were observed. Considering that the first step of the synthesis was quantitative, that two methylenes were lost during this step, and that the yield of the ^{14}C-DADN step was 88%, also considering that a yield of 91% was obtained for the HMX preparation, 1.05 mCi of activity should theoretically be obtained for the HMX sample. Since we were not able to reproduce the yields obtained with cold materials with the radioactive materials, the overall yield for ^{14}C-HMX synthesis was 79%, which is lower than the Bachmann process but this route yielded a pure product which needed no further separation.

Liquid scintillation counting revealed a total activity of 675μCi for our ^{14}C-HMX sample indicating that the original radioactivity of the starting material was not 2 mCi, but 1.3 mCi. Nevertheless, the ^{14}C-HMX, even with a total activity of 675 μCi, had a level of radioactivity high enough to be used in the biodegradation study.

4.2 Synthesis of ^{15}N-RDX

In the current ^{15}N-RDX synthesis, formaldehyde (37%) was reacted with isotopic nitrogen-15 concentrated ammonium hydroxide to yield ^{15}N-HMTA quantitatively. Nitration of this product yielded ^{15}N-RDX (56%) and the infrared and NMR spectroscopy of the product was identical to those of a commercial sample. The yields for the two steps of the synthesis were identical to those described in the literature (Ref.16). The opening of hexamethylenetetramine resulted in the loss of three carbons and one nitrogen. In the synthesis of ^{14}C-RDX, it was dramatic to loose the radioactive carbons and, since the labelled starting materials was mixed with cold materials, the chance of retrieving carbon 14 in the cycle was statistic. In the case of ^{15}N-RDX, the concentrated ammonium hydroxide is fully nitrogen-15 labelled so, even if a nitrogen is lost during the opening of ^{15}N-HMTA, all the nitrogen retrieved in the RDX cycles are labelled. This product was flegmatized with 8% ethanol and sent to the Biotechnology Research Institute (BRI). The ethanol was evaporated before introducing the labelled RDX into the microcosm experiments.

5.0 CONCLUSION

The synthesis of carbon-14 labelled HMX was performed successfully. In all cases, the IR and NMR spectroscopy of the ^{14}C-products were identical to that of known samples. ^{14}C-HMX was synthesized according to the DADN route using the nitric acid/phosphorus pentoxide system for the nitrolysis of DADN. Many routes were evaluated during this study with cold starting materials and the DADN procedure was considered to be the best method to synthesize radioactive HMX. It appeared that the nitric acid strength, phosphorus pentoxide concentration and temperature were very important parameters of the nitrolysis reaction. Best results and best yields were obtained using freshly distilled nitric acid 100%. The ^{14}C-HMX sample had a total activity of 675 μCi. Considering the yields and the fact that some methylene groups are lost during synthesis, the starting ^{14}C-formaldehyde material had a total activity of 1.3 MCi. The purity of all the products isolated in the syntheses was monitored by rigorous control of their IR and NMR spectroscopy. The synthesis of ^{15}N-RDX was performed according to the Hale process and identical yields were obtained for the two steps of the synthesis. The IR and NMR spectroscopy revealed that the ^{15}N-RDX sample was

identical to a commercial known compound. All the nitrogens within the RDX cycles were labelled with nitrogen-15.

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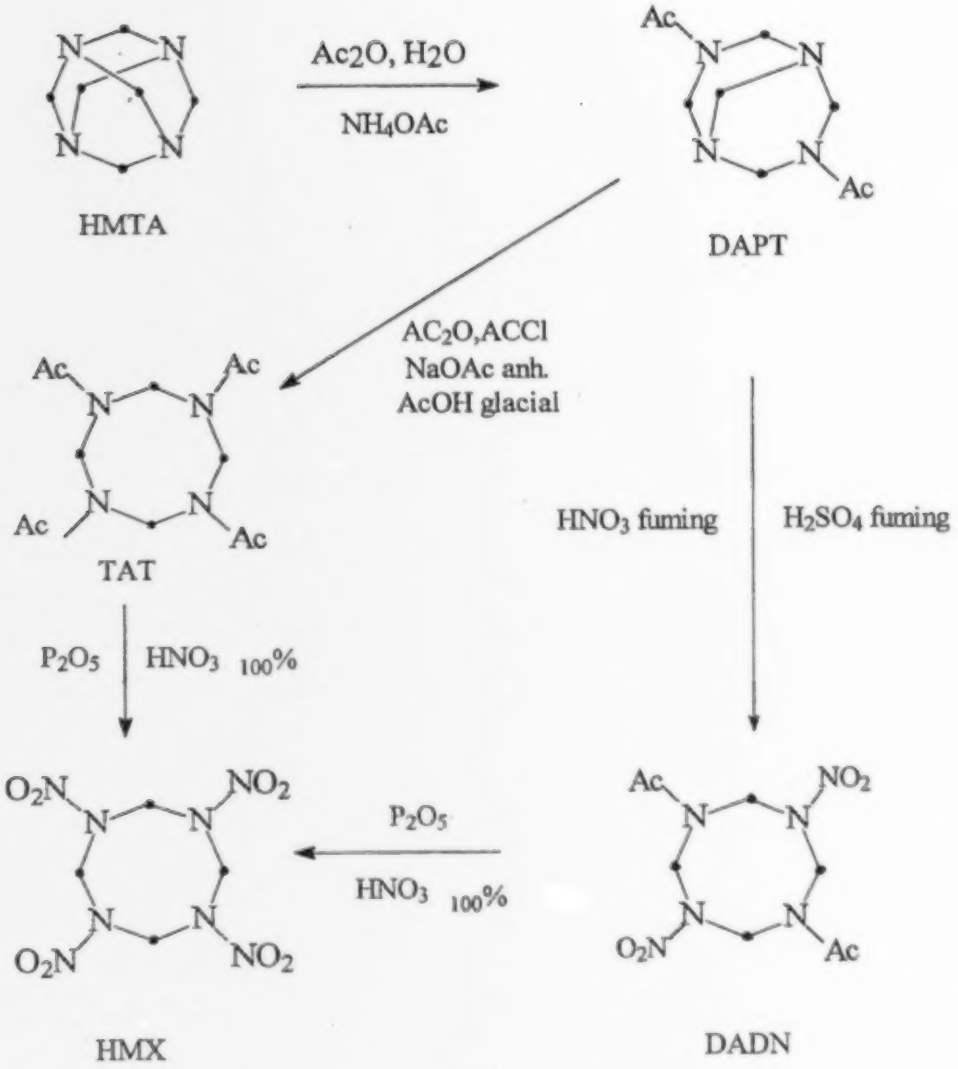

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Within the framework of an R & D project on bioremediation of soils contaminated with energetic compounds, the biodegradation of energetic nitramine products such as octogen (HMX) and hexogen (RDX) is under study. Microcosm assays can be performed with radioactive carbon-14 labelled substrates or with isotopic nitrogen-15 labelled substrates in order to ascertain and monitor their biodegradation pathways and to optimize parameters for a bioremediation process. To synthesize ^{14}C-HMX, acetylation of labelled hexamethylenetetramine (^{14}C-HMTA) was done yielding 3,7-diacetyl-1,3,5,7-tetraazabicyclo-[3.3.1]-nonane(^{14}C-DAPT) which was nitrated to obtain 1,5-diacetyloctahydro-3,7-dinitro-a,3,5,7-tetrazocine (^{14}C-DADN) in one step. Nitrolysis of ^{14}C-DADN was achieved using a mixture of 100% nitric acid and phosphorus pentoxide to yield ^{14}C-HMX. The synthesis of this carbon-14 labelled HMX was optimized first using cold starting materials and then conducted with labelled compounds. To synthesize ^{15}N-RDX, nitration of nitrogen-15 hexamethylenetetramine (^{15}N-HMTA) was done according to the Hale Process. ^{15}N-HMTA was prepared by reaching cold formaldehyde with isotopic nitrogen-15 ammonium hydroxide.

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